The combinatorial synthesis and chemical and biological evaluation of a 1,4-benzodiazepine library

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ABSTRACT   A library of 192 structurally diverse 1,4-benzodiazepine derivatives containing a variety of chemical functionalities including amides, carboxylic acids, amines, phenols, and indoles was constructed from three components, 2-aminobenzophenones, amino acids, and alkylating agents, by employing Geysen's pin apparatus [Geysen, H. M., Rodda, S. J., Mason, T. J., Tribbick, G. & Schoofs, P. G. (1987) *J. Immunol. Methods* 102, 259–274]. Rigorous analytical verification of the chemical integrity and yield of a representative collection of the diverse derivatives was carried out. In addition, the library of derivatives was evaluated for binding to the cholecystokinin A receptor by employing a competitive radioligand binding assay. This provided detailed structure versus activity relationships that were confirmed by independent large-scale synthesis and evaluation of several of the 1,4-benzodiazepine derivatives.

An essential early step in the development of therapeutic agents continues to be the identification of lead compounds that interact with the receptor or enzyme target of interest. Many analogs of the lead compounds are then constructed to define the key recognition elements for maximal activity. In almost all cases, the initial lead compounds are identified by the target-based screening of large collections of compounds and/or biological extracts. Unfortunately, despite recent technological advances that have greatly expedited screening procedures to allow hundreds of thousands to millions of compounds to be efficiently evaluated (1), target-based screening often does not result in the identification of suitable lead compounds due to the limited size and inadequate structural diversity of the collections of compounds that are being evaluated.

Very powerful chemical and biological methods have recently been developed for the generation of large combinatorial libraries of peptides and oligonucleotides that are then screened against a specific receptor or enzyme to determine the important structural features of the biopolymer for binding to that receptor or enzyme (2, 3). By employing these methods, numerous peptide and oligonucleotide ligands have been identified to diverse receptor targets, clearly demonstrating the power of combinatorial synthesis and screening strategies. Unfortunately, peptides and oligonucleotides generally have very poor oral activities, rapid *in vivo* clearing times, and thus have limited utility as bioavailable therapeutic agents. The combinatorial synthesis and screening of bioavailable organic compounds would be a powerful extension of this approach. Toward this goal, the combinatorial synthesis and evaluation of nonbiological oligoamides (4) and oligocarbamates (5) as potential therapeutic agents have recently been reported. We have reported (6) a general method for the expedient solid-phase synthesis of 1,4-benzodiazepine derivatives, one of the most important classes of bioavailable therapeutic agents with widespread

biological activities including anxiolytic, anticonvulsant, and antihypnotic activities (7), cholecystokinin (CCK) receptor A and receptor B antagonists (8), opioid receptor ligands (9), platelet-activating factor antagonists (10), human immunodeficiency virus trans-activator Tat antagonists (11), GP-IIbIIIa inhibitors (12), reverse transcriptase inhibitors (13), and ras farnesyltransferase inhibitors (14). Here we describe the combinatorial synthesis and rigorous chemical and biological evaluation of a library of 192 structurally diverse and spatially separate 1,4-benzodiazepine derivatives.

MATERIALS AND METHODS

Reagents and General Methods. 9-Fluorenylmethoxycarbonyl (Fmoc)-protected amine-derivatized pins were purchased from Cambridge Research Biochemicals, Fmoc-protected amino acids and 4-hydroxymethylphenoxyacetic acid were purchased from Nova Biochem (currently available from Chiron), and all other reagents and solvents were purchased from Aldrich. The following Fmoc-amino acids were employed: Fmoc-Ala-OH, Fmoc-Asp(O-*t*-Bu)-OH, Fmoc-Val-OH, Fmoc-Gly-OH, Fmoc-Nle-OH, Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc-Lys(Boc)-OH, Fmoc-Trp(Boc)-OH, Fmoc-D-Ala-OH, Fmoc-D-Phe-OH, and Fmoc-D-Trp(Boc)-OH, where *t*-Bu is *tert*-butyl, Nle is norleucine, and Boc is *t*-butoxycarbonyl. For the alkylation reactions, benzyl and xylyl bromides and methyl, ethyl, propyl, acetamidyl, and heptyl iodides were employed. Chemical synthesis was performed in chemically resistant polypropylene deep-well microtiter plates purchased from Beckman (catalog no. 267006). ^{125}I-labeled Bolton–Hunter reagent-labeled CCK octapeptide was purchased from NEN (catalog no. NEX-203).

Combinatorial Benzodiazepine Synthesis. Hydroxy-functionalized 2-aminobenzophenone derivatives 1a and 1b were coupled to the 4-hydroxymethylphenoxyacetic acid-cleavable linker by deprotonation with 1 equivalent of potassium bis(trimethylsilyl)amide followed by alkylation with allyl 4-bromomethylphenoxyacetate (Fig. 1) (6). Subsequent treatment with FmocCl and pyridine in CH$_2$Cl$_2$ resulted in protection of the aniline functionality to provide 2a and 2b. Removal of the allyl protecting group was then accomplished with SnBu$_3$H and Pd(PPh$_3$)$_4$ as catalyst to provide 3a and 3b ready for coupling to the amine-derivatized polyethylene pins.

The Fmoc-protected amine-derivatized pins were rinsed with CH$_2$Cl$_2$, MeOH (air-dried for 10 min), and N,N-dimethylformamide (DMF) and then immersed in 20% (vol/vol) piperidine in DMF (once for 1 min and then once for 20 min). After rinses with DMF, MeOH (air-dried), and DMF (twice), the pins were immersed for 12 h at room temperature in a DMF stock solution, which was 0.05 M in acid 3a or 3b

Abbreviations: Fmoc, 9-fluorenylmethoxycarbonyl; Boc, *t*-butoxycarbonyl; CCK, cholecystokinin; DMF, N,N-dimethylformamide; THF, tetrahydrofuran.
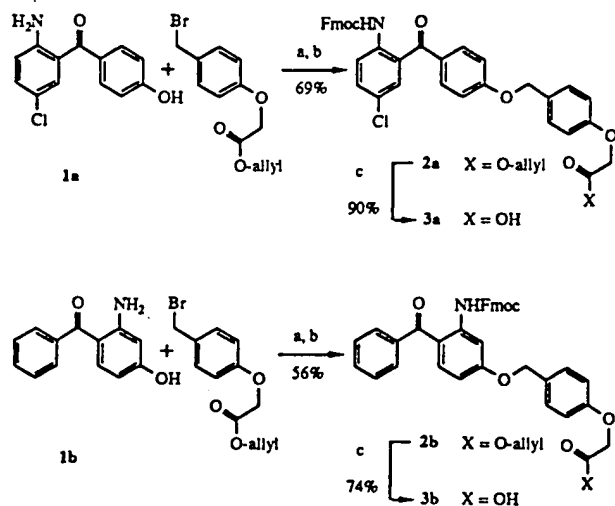*To whom reprint requests should be addressed.

FIG. 1. Attachment of 2-aminobenzophenones to the acid-cleavable linker. Steps: a, KN(SiMe₃)₂ in DMF; b, FmocCl pyridine in CH₂Cl₂; c, Pd[PPh₃]₄ SnBu₃H in CH₂Cl₂.

and 0.055 M in hydroxybenzotriazole and diisopropylcarbodiimide. The derivatized pins were then rinsed with DMF (twice), MeOH (air-dried), CH₂Cl₂ (twice), and DMF followed by treatment with 20% piperidine in DMF (once for 1 min and then once for 20 min) to provide 4 (Fig. 2). The resulting pins were then rinsed with DMF (three times), MeOH (air-dried), and CH₂Cl₂ (twice) and then immersed in a 0.2 M solution of the appropriate Fmoc-protected amino acid fluoride in CH₂Cl₂ to provide anilide 5. To obtain high yields in the coupling step, the Fmoc-amino acid fluorides should be prepared employing cyanuric fluoride (15), and the workup should include extraction with dilute aqueous bicarbonate (three times) and 1 M aqueous sodium bisulfate (three times) to remove any cyanuric fluoride byproducts (further purification is not necessary). The coupling reaction was allowed to continue for 3 days to ensure complete coupling of the most hindered amino acid derivatives, for example, valine or isoleucine. To prevent evaporation of the solvent, the pins immersed in the wells of the Beckman microtiter plate were maintained in a CH₂Cl₂-saturated atmosphere by placing the plate in a dessicator containing a layer of CH₂Cl₂. The pins were then rinsed with CH₂Cl₂ (three times), MeOH (air-dried), and DMF (twice), followed by treatment with 20%
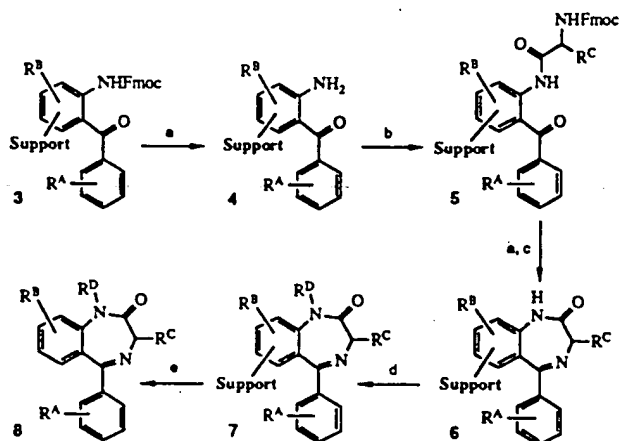

FIG. 2. 1,4-Benzodiazepine synthesis on solid support. a, 20% piperidine in DMF; b, N-Fmoc-amino acid fluoride in CH₂Cl₂; c, 5% acetic acid in DMF; d, lithiated 5-phenylmethyl-2-oxazolidinone in DMF/THF, 1:10 (vol/vol), followed by alkylating agent in DMF; e, trifluoroacetic acid/H₂O/Me₂S, 95:5:10 (vol/vol).

piperidine in DMF (once for 1 min and then once for 20 min) to remove the Fmoc protecting group. After rinsing with DMF, MeOH (air-dried), and again DMF (twice), the pins were immersed in 5% (vol/vol) acetic acid in butanol or DMF at 60–80°C for 12 h to provide the cyclic product 6. The pins were then rinsed with DMF (three times) and tetrahydrofuran (THF) (twice). The pins and all necessary reagents were transferred to a glove bag preflushed with N₂ (g). After a final THF rinse, the pins were immersed in 0.12 M lithiated 4-phenylmethyl-2-oxazolidinone in THF containing 10% DMF at 0°C for 30 min. The pins were then immersed, without rinsing, in a 0.40 M solution of the alkylating agent in DMF that had been prepared immediately before alkylation. After 1 h at 0°C, the alkylation sequence was repeated and then allowed to warm to room temperature over several hours. The pins were removed from the glove bag, rinsed with DMF, DMF/H₂O, MeOH (air-dried), and CH₂Cl₂, and cleaved from the support by immersion in 85:10:5 (vol/v 1) trifluoroacetic acid/dimethyl sulfide/H₂O for 24 h. For benzodiazepine derivatives incorporating tryptophan, 85:5:5:5 (vol/vol) trifluoroacetic acid/dimethyl sulfide/H₂O/1,2-ethanedithiol was employed as the cleavage mixture to prevent oxidative decomposition of the indole ring (16). The cleavage mixture was then removed with a Jouan RC10.10 concentrator equipped with a microtiter-plate rotor so that the free 1,4-benzodiazepine derivatives were spatially separated in the individual wells of the microtiter plate.

Analytical Evaluation of the 1,4-Benzodiazepine Library. Out of the library of 192 benzodiazepine derivatives, 28 unique derivatives were evaluated by reverse-phase HPLC analysis using a Rainin C₁₈ column, a 15–100% gradient of methanol in water buffered with 0.1% trifluoroacetic acid, and UV detection at 350 nm. The compound corresponding to the major peak was isolated and subjected to fast atom bombardment mass spectrometric analysis to verify the structure of the benzodiazepine derivative. Sixteen of the compounds were derived from aminobenzophenones 1a or 1b, valine, and each of the eight alkylating agents employed in the library, and 12 of the compounds were derived from aminobenzophenone 1a, each of the 12 amino acids that were employed in the library, and ethyl iodide.

In addition, for the eight benzodiazepine derivatives c n-structed from 2-aminobenzophenone 1a, valine, and each of the eight alkylating agents and for the 12 benzodiazepine derivatives constructed from 2-aminobenzophenone 1a, each of the 12 amino acids, and ethyl iodide, 200 μl of a st ck solution of fluorenone (4 mg/ml) in DMF was added, followed by reverse-phase HPLC analysis to determine the relative peak area of the 1,4-benzodiazepine derivative to the fluorenone standard as monitored at 350 nm. The quantity of material produced per pin was then calculated from the extinction coefficients of the derivatives that were determined on material prepared on large scale (data not shown).

Racemization of the amino acid stereocenter during the synthesis sequence was also evaluated for 1,4-benzodiazepines, prepared from 2-aminobenzophenone 1a, both D- and L-alanine, and ethyl iodide, by etherification of the 1,4-benzodiazepine derivatives with excess diazomethane in 1:1 (vol/vol) ethyl acetate/MeOH for 12 h. No racemization (<2%) was detected by HPLC analysis on a 10 mm × 25 cm covalent D-dinitrobenzoylphenylglycine chiral pirkle column from Regis (Morton Grove, IL), with 4% (vol/vol) isopropanol in hexane as the eluent, a flow rate of 6 ml/min, and absorbance monitored at 260 nm (17). The (S)-derivative was eluted at 19.4 min and the (R)-derivative was eluted at 21.4 min.

Competitive Radioligand Binding Assay t CCK A Recept r. Assays were performed according to established procedures with minimal modification (18, 19). Fresh rat pancreas (500 mg) cleaned of extraneous tissue in 50 parts of Hepes buffer

1 (10 mM Hepes/130 mM NaCl/5 mM MgCl$_2$, pH 7.4) at 0°C was homogenized (twice for 15 sec) with a Tekmar (Cincinnati) Tissumizer, model TR10, at setting 6. The tissue was then isolated by centrifugation at 20,000 × g for 20 min at 4°C and was resuspended in 500 ml of Hepes buffer 2 [10 mM Hepes/130 mM NaCl/5 mM MgCl$_2$, pH 7.4/phenylmethylsulfonyl fluoride (1 mg/liter)/bacitracin (200 mg/liter)] at 0°C. The assays, which were performed simultaneously in a microtiter plate format, were initiated by combining the appropriate quantity of a stock solution of 1,4-benzodiazepine in 1:1 (vol/vol) dimethyl sulfoxide/H$_2$O, tissue homogenate (400 μl), and ^{125}I-labeled Bolton–Hunter-labeled CCK octapeptide (1.7 μl, 1.6 fmol). Samples were then incubated for 40 min at 25°C followed by filtration through Whatman GF/B filter paper, presoaked in Hepes buffer 1 at 0°C, employing a Bio-Rad Bio-Dot microfiltration apparatus. The filtration wells were rapidly rinsed with ice-cold Hepes buffer 1 (twice with 200 μl). The filter paper was air-dried and then evaluated employing a Molecular Dynamics Phosphor-Imager.

RESULTS

Synthesis and Screening Strategy. In the construction and evaluation of a combinatorial library of 1,4-benzodiazepine derivatives, we felt that several key criteria should be met. (*i*) The benzodiazepine derivatives should be synthesized on a solid support because the solid support strategy provides facile isolation of reaction products from reaction mixtures, thus enabling one to drive reactions to completion by the use of excess reagents. (*ii*) After synthesis of the compounds is complete, the compounds should be removed from the support so that the compounds can be assayed in solution. While highly sophisticated assays for solubilized receptor or enzyme targets have been developed for support-bound material (20–22), whole-cell assays require soluble ligands. In addition, the solid support may complicate or interfere with receptor binding to the support-bound small molecule. (*iii*) Initially, in the construction of the library, the compounds should be synthesized in a spatially separate fashion to enable rigorous chemical and biological characterization of the library. While solid-phase peptide and oligonucleotide synthesis is highly developed, general methods for the solid-phase synthesis of organic compounds have seen only limited development (23, 24). The capability to determine the yield and chemical integrity of the structurally diverse derivatives is, therefore, an essential requirement. In addition, by maintaining compounds in a spatially separate fashion, biological evaluation provides detailed structure versus activity relationships that cannot readily be obtained employing pools of compounds. (*iv*) The final criteria in choosing a strategy for the construction of a library of organic compounds was to employ a strategy that did not require the development of costly instrumentation and, rather, to rely on instrumentation that had already been developed for preexisting high-throughput screening procedures.

According to these criteria we have chosen to construct the 1,4-benzodiazepine derivatives from three readily available components, 2-aminobenzophenones, amino acids, and alkylating agents, providing access to a large number of structurally diverse derivatives (*vida infra*) (6). In constructing the library, we have also chosen to employ Geysen's pin apparatus developed for peptide epitope mapping (25). Geysen's apparatus is configured such that 96 polyethylene pins are attached to a supporting block such that each pin fits into a well of a 96-well microtiter plate. The pins are prederivatized with aminoalkyl groups providing sites for substrate attachment. Even though each well of the microtiter plate serves as a distinct reaction vessel for performing chemical reactions, each synthesis step can rapidly and efficiently be performed

on multiple pins in a simultaneous fashion by employing preexisting microtiter-plate-based instrumentation that has been extensively developed predominately for screening procedures. Approximately 100 nmol of material is produced per pin, which is sufficient for multiple biological assays and for analytical evaluation of the purity and chemical integrity of the individual compounds.

Chemical Synthesis of the Combinatorial Library. The combinatorial library of 192 structurally diverse benzodiazepine derivatives was constructed according to the synthesis methods as reported (6). The 2-aminobenzophenones **1a** and **1b** were first coupled to the pin support through the acid-cleavable linker 4-hydroxymethylphenoxyacetic acid as shown in Fig. 1. Solid-supported synthesis of the benzo°i-azepine derivatives was then accomplished as shown in Fig. 2. After removal of the Fmoc protecting group, the support-bound aminobenzophenones **4** were coupled with the appropriate amino acid by treatment with a stock solution of the corresponding Fmoc-protected amino acid fluoride. After removal of the Fmoc-protecting group from **5**, cyclization was accomplished to provide **6** by addition of 5% acetic acid in DMF. The support-bound benzodiazepines **6** were then deprotonated and, subsequently, alkylated by transferring to a stock solution of alkylating agent. We have found that by repeating the alkylation sequence twice, no unalkylated benzodiazepine was detected (<2%) as determined by HPLC analysis after cleavage of the product from the support (*vida infra*). The fully functionalized benzodiazepine **7** was removed from the pin support by treatment with trifluoroacetic acid. The final benzodiazepine products **8** were then readily isolated by concentration employing a microtiter-based solvent evaporator to provide the benzodiazepines in a microtiter format ready for analytical or biological evaluation. In the construction of the benzodiazepine library, a variety of chemical functionalities were introduced through the amino acid side chains including carboxylic acid, amine, and indole functionality. Both activated and unactivated alkylating agents and the functionalized alkylating agent iodoacetamide were also included in the library such that the library contained both a variety of chemical functionality and diverse steric properties.

Analytical Evaluation of the Combinatorial Library. To evaluate the chemical integrity of the combinatorial library, two analytical methods were employed. (*i*) The chemical structures of 28 of the structurally diverse benzodiazepine derivatives were confirmed by fast atom bombardment mass spectrometry of the compound corresponding to the major UV active peak (in most cases the only peak) as identified by reverse-phase HPLC analysis. Each of the 2-aminobenzophenones, amino acids, and alkylating agents were incorporated into at least one of the 28 benzodiazepine derivatives, indicating that all of the derivatives of the three synthesis components are compatible with the general solid-phase synthesis sequence. (*ii*) For 20 unique derivatives, where again each of the 2-aminobenzophenones, amino acids, and alkylating agents were incorporated into at least one of the derivatives, a set volume of a stock solution containing fluorenone as an internal standard was added followed by HPLC analysis. The quantity of material produced per pin then was determined directly by correlating the peak area of the benzodiazepine derivative relative to the internal standard with the relative extinction coefficients of the different 1,4-benzodiazepine derivatives. Per pin, the average amount of 1,4-benzodiazepine produced was 86 nmol (theoretical yield is 100 nmol). The 1,4-benzodiazepine prepared from aminobenzophenone **1a**, isoleucine, and ethyl iodide provided the least amount of material (67 nmol). Finally, no racemization of the benzodiazepine derivatives prepared from 2-aminobenzophenone **1a**, L- and D-alanine, and ethyl iodide was observed as determined by chiral HPLC analysis.

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Biological Evaluation of the 1,4-Benzodiazepine Library. In addition to rigorously demonstrating the chemical integrity of the library, we also wanted to demonstrate that the library could be evaluated reliably to obtain detailed structure versus activity relationships against important receptor and enzyme targets. As the initial target, we chose to assay the library for ligands to the CCK A receptor for several reasons. (*i*) The CCK A receptor has wide-ranging biological functions with potential therapeutic applications (26). (*ii*) Researchers at Merck have identified a number of 1,4-benzodiazepine derivatives that bind to this receptor (27). (*iii*) The competitive radioligand binding assay for this receptor target is amenable to high throughput. The results of the simultaneous evaluation of all 192 benzodiazepines in the library employing the competitive radioligand binding assay in a microtiter format with the ligands at 30 μM are shown in Fig. 3. In the preliminary screen shown, clearly the benzodiazepine derivatives that incorporated either D- or L-tryptophan bound much more tightly than the benzodiazepine derivatives that incorporated other amino acids. A second evaluation was then performed on the 32 benzodiazepines that incorporated D- or L-tryptophan at 7.5 μM, 3.0 μM, 1.0 μM, 0.3 μM, 0.1 μM, and 0.03 μM (data not shown). The data from these experiments clearly demonstrated two additional levels of structural discrimination, wherein benzodiazepines incorporating 2-aminobenzophenone **1b** showed significantly higher affinity than benzodiazepines incorporating 2-aminobenzophenone **1a**, and benzodiazepines incorporating D-tryptophan showed higher affinity than benzodiazepines incorporating L-tryptophan. Finally, in accord with Merck's data, introduction of different alkyl groups R^C had a more modest effect on binding affinity.

To confirm the assay results obtained by evaluation of the library, a selected number of benzodiazepine derivatives from the library were synthesized on a large scale and purified, and the IC_{50} values were determined exactly by performing the assays in triplicate relative to a known inhibitor, compound **9**, developed by researchers at Merck (27) (Table 1). The relative IC_{50} values for the selected benzodiazepine derivatives directly parallel the relative binding affinities that were observed in assaying the benzodiazepine library thus validating the combinatorial synthesis and screening strategy as a powerful method for the rapid col-

Table 1. CCK A receptor binding affinities for selected 1,4-benzodiazepines **8** (Fig. 2)

Compound	R^A	R^B	R^C	R^D	IC_{50}, μM
8a	4'-OH	5-Cl	CH_3	H	>30
8b	4'-OH	5-Cl	$(CH_3)_2CH$	H	>30
8c	4'-OH	5-Cl	$(CH_3)_2CH$	$PhCH_2$	>30
8d	4'-OH	5-Cl	3-Indolyl-CH_2	CH_3CH_2	2.1 ± 0.1
8e	4'-OH	5-Cl	3-Indolyl-CH_2	CH_3CH_2	11 ± 4
8f	—	4-OH	3-Indolyl-CH_2	CH_3CH_2	0.08 ± 0.01
9	—	—	3-Indolyl-CH_2	H	0.61 ± 0.15
10	—	—	3-Indolyl-CH_2	CH_3CH_2	0.12 ± 0.02

All compounds were characterized by ^{1}H NMR, ^{13}C NMR, and fast atom bombardment mass spectrometry. Receptor binding affinities are expressed as the IC_{50} (mean ± SD), the concentration of compound required for half-maximal inhibition of binding of ^{125}I-labeled CCK-8 to CCK A receptors, were determined at six concentrations with the experiments performed in triplicate. All compounds have the (*R*)-configuration except for compound **8e**, which has the (*S*)-configuration. Researchers at Merck have determined (27) that compound **9** had an IC_{50} value of 1.2 μM for CCK A receptors by employing ^{125}I-labeled CCK-33.

lection of detailed structure versus activity data. In addition, it should be noted that introduction of a hydroxyl functionality on the 2-aminobenzophenone to provide a site of attachment to the solid support did not result in unfavorable interactions with the receptor target as long as it was appropriately situated (28). Thus, while benzodiazepines incorporating the 2-aminobenzophenone **1a** showed lower affinity to CCK A receptor than compounds incorporating an unsubstituted 2-aminobenzophenone, benzodiazepine derivatives incorporating 2-aminobenzophenone **1b** showed at least as high affinity to CCK A receptor. Unfavorable interactions of the appended hydroxyl functionality can, therefore, be minimized by introducing the hydroxyl functionality at different sites on different 2-aminobenzophenone derivatives, while contributing to greater library diversity.

DISCUSSION

A library of 192 structurally diverse 1,4-benzodiazepine derivatives containing a variety of chemical functionalities including amides, carboxylic acids, amines, phenols, and
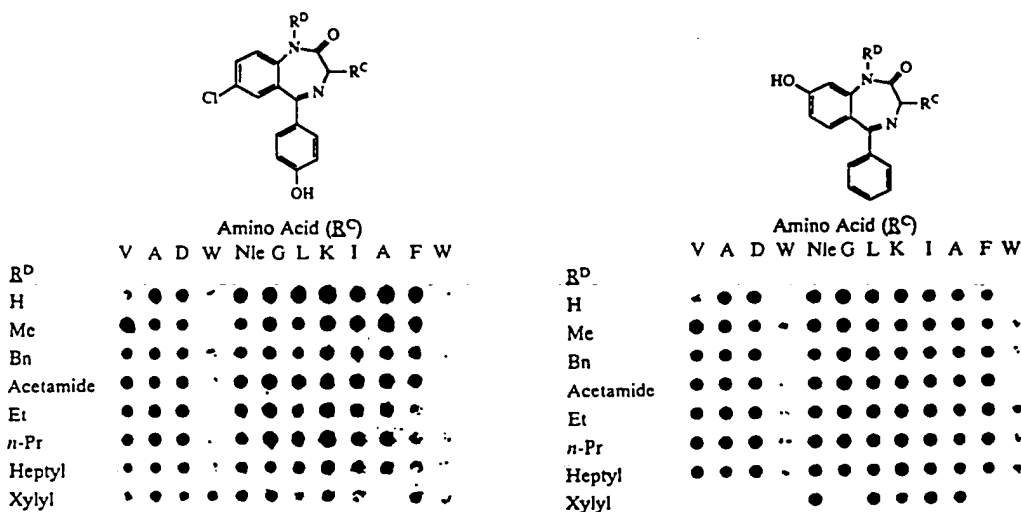

FIG. 3. Receptor binding affinity of 1,4-benzodiazepine derivatives. Receptor binding affinity of the 1,4-benzodiazepine derivatives at 7.5 μM is monitored by the percent displacement of ^{125}I-labeled CCK-8 from the CCK A receptors as observed by the relative signal intensity upon PhosphorImager exposure. Group R^C corresponds to the side chain of the amino acid that is incorporated into the benzodiazepine structure. The absolute configuration for most compounds is *S*, except for the derivatives in the last three columns (incorporating amino acids A, F, and W), where the absolute configuration is *R*. Nle, norleucine; Bn, benzyl; *n*-Pr, *n*-propyl. In the receptor binding assay, the two benzodiazepine derivatives prepared from valine with R^C = H were replaced with compound **9** (Table 1).

indoles has been constructed in a combinatorial fashion from three components, 2-aminobenzophenones, amino acids, and alkylating agents, by employing Geysen's pin apparatus. Rigorous analytical verification of the chemical integrity and yield of a representative collection of the diverse derivatives has also been accomplished. In addition, the library of derivatives was evaluated for binding to the CCK receptor by employing a simultaneous competitive radioligand binding assay. This provided detailed structure versus activity relationships that were confirmed by independent large-scale synthesis and evaluation of several of the derivatives.

By employing the described protocols and reaction conditions, a greatly expanded library could rapidly be accessed by including the >40 commercially available preprotected natural and unnatural amino acids that are compatible with our solid-phase synthesis protocols.[†] In addition, >50 alkylating agents are available commercially, many of which have already been evaluated with our solid-phase synthesis strategy, including cinnamyl bromide derivatives, iodoacetonitrile, and a range of iodoalkanes, alkenes, and alkynes. Furthermore, we have developed (unpublished data) a method to rapidly and efficiently construct on solid-support numerous structurally diverse 2-aminobenzophenone derivatives incorporating a variety of chemical functionalities from commercially available precursor molecules. Geysen has reported (25) that 2000 unique hexapeptide derivatives could be constructed in <2 weeks by employing his pin apparatus. Considering that more than twice as many chemical steps are required to synthesize a hexapeptide than are necessary to synthesize a 1,4-benzodiazepine according to our synthesis strategy, the construction of >10,000 benzodiazepine derivatives should certainly be feasible.

The results reported in this article indicate that the combinatorial synthesis of nonpolymeric organic molecules involving multistep reaction sequences and incorporating a variety of sensitive chemical functionalities will provide effective and reliable access to compound libraries for the identification of lead structures and for the rapid determination of structure versus activity relationships. The principles and methods outlined in this article should be applicable to a number of other therapeutically important classes of organic compounds and designed recognition elements.

[†]For uniformly high yields in the synthesis of benzodiazepine derivatives incorporating amino acids with side-chain functionality, for example, Asp, Glu, Lys, Tyr, Asn, Gln, Met, Ser, and Trp, 2,6-di-*t*-butylpyridine should be included in the acid fluoride coupling step (6).

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